

RESEARCH ARTICLE



Dihydromyricetin improves vascular hyporesponsiveness in experimental sepsis via attenuating the over-excited MaxiK and K_{ATP} channels

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ABSTRACT

Context: Dihydromyricetin (DMY) has oxidation resistance, anti-inflammatory and free radical scavenging capabilities. The preventive effects of DMY for vascular hyporeactivity remain unclear.

Objective: This study investigates the preventive effects of DMY in vascular hyporeactivity.

Materials and methods: The experimental sepsis was induced by transvenous administration of lipopolysaccharide (LPS) to Sprague–Dawley (SD) rats. DMY-treated rats received daily administration of DMY, 5 µg/kg dissolved in DMSO through the tail vein for 7 days. The invasive mean arterial pressure (MAP) of the caudal ventral artery was measured. Dose-response curves for norepinephrine (NE, doses from 10^{-9} to 10^{-6} M) were obtained in isolated thoracic aorta in a cumulative manner. The function of MaxiK and K_{ATP} channels were investigated using whole-cell patch clamp recording. The Elisa was adopted to measure the serum concentration of NO, MDA, 3-NT, IL-1β and TNF-α.

Results: The increased MAP in septic rats induced by vasopressor agents was smaller than that in control rats. However, the % of increased MAP induced by vasopressor agents was raised by DMY injection (NE: 20.4 ± 8.495 vs. $15.16 \pm 5.195\%$; AVP: 14.05 ± 2.459 vs. $9.583 \pm 2.982\%$, $p < 0.05$). The vascular hyporesponsiveness to NE (10^{-6} M) *in vitro* was increased by 51% in LPS + DMY group compared with that in LPS + Con group (2.74 ± 0.81 vs. 1.82 ± 0.92 g, $p < 0.05$). Charybdotoxin (a potent MaxiK channel blocker) and glibenclamide (a K_{ATP} channel blocker) pretreatment, instead of 4-aminopyridine (4-AP) and BaCl₂, could diminish the DMY-induced improvement of vasoconstrictor hyporeactivity (ChTX: 73.2 ± 11.8 vs. $71.8 \pm 13.5\%$; Glib: 63.1 ± 12.5 vs. $58.1 \pm 13.7\%$, $p > 0.05$). DMY blunted the highly sensitized MaxiK and K_{ATP} channels of arterial smooth muscle cells isolated from the thoracic aorta of LPS rats. DMY decreased the serum level of NO, MDA, IL-1β and TNF-α, which had increased in LPS rats.

Discussion and conclusions: Our results indicate that DMY administration ameliorated the impaired contractility of the rat aorta in experimental sepsis. Such an effect is mediated by normalization of the over-excited MaxiK and K_{ATP} channels possibly via oxidative stress inhibition.

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Introduction

Sepsis, often presenting with multiple organ dysfunction syndrome and organ failure, is a major problem that causes the most challenging efforts in intensive care today (Johnson and Mayers 2001). One of the most important clinical characteristics of sepsis and septic shock is the vascular hyporesponsivity to vasopressor agents (Donaldson and Myers 1996; Strunk et al. 2001). It represents an important condition for patient survival. Different therapeutic strategies aim to improve vital organ function (Leone and Martin 2008). The identification of new intracellular signalling related to sepsis progression might contribute to the development of therapeutic strategies to reduce sepsis-associated mortality.

The exact mechanism underlying the susceptibility of hypotension in patients with sepsis remains unclear. However, oxidative stress is associated with impaired vasoconstriction in sepsis (Szabo et al. 1995; Wu et al. 2004). According to Wu et al. (2004), antioxidant treatment, before cecal ligation and puncture (CLP) surgery to induce sepsis, increases mice survival and decreases hypotension, plasma NO metabolites, oxidative stress, NOS2 mRNA and angiotensin II (AngII) hyporesponsivity. In

regard to endothelial cells, Huang et al. (2016) showed that lipopolysaccharide (LPS) treatment of human umbilical vein endothelial cells increases oxidative stress, malondialdehyde levels, superoxide dismutase 2 protein expression and phosphorylation of c-Jun N-terminal kinases, and decreases SOD1 expression. Furthermore, potassium channels in vascular smooth muscle cells (VSMCs), which are widely distributed in vasculatures, are reported to play an important role in the vascular tone regulation under pathophysiological conditions. Using patch-clamp techniques, Dopico et al. (2002) found that administration of large-conductance Ca^{2+} -activated K^{+} (BK_{Ca}, MaxiK, Slo1) channel blockers could eliminate vasodilation. Ample evidence from experimental animal models and the identification of K_{ATP} channel mutations in patients also indicate that K_{ATP} channel plays a critical role in vascular tone regulation (Shi et al. 2012).

Dihydromyricetin (DMY), a flavonoid compound, is extracted from the stems and leaves of *Ampelopsis grossedentata* (Hand.-Mazz.) W.T. Wang (Vitaceae). *Ampelopsis* is widely distributed in tropical and subtropical regions of the world. It is used in Chinese traditional medicine for treating liver disorders (Pang et al. 2011; Liu et al. 2017). Previous studies documented

that DMY has oxidation resistance, anti-inflammatory, free radical scavenging capabilities among other biological activities (Zhang et al. 2003). The effects of DMY on LPS-induced vascular hyporesponsivity to vasopressor agents, has not been reported. This study explores the effects of DMY on vascular hyporesponsivity in an experimental sepsis model by focusing special attention on the role of VSMCs MaxiK and K_{ATP} channels in freshly isolated rat thoracic aorta.

Materials and methods

Animals

A total of 72 pathogen-free, adult male Sprague–Dawley rats (weighing 200–250 g) were obtained from the Shanghai Slac Experimental Animal Centre (Shanghai, China). The rats were housed in individual cages in a temperature-controlled room with alternating 12 h light/dark cycles. Food was withheld 8 h before the start of experiments, but all animals had free access to water. The study was approved by the Animal Care Committee of the Binzhou Medical College and performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Experimental design and sample collection

The experimental animals were randomly divided into four groups of 18 rats each: normal saline-injected rats treated with DMSO group as control (Con + NS), LPS-injected rats treated with DMSO group (LPS + NS), control rats treated with DMY (Sigma-Aldrich, St. Louis, MO), group (Con + DMY) and LPS rats treated with DMY group (LPS + DMY). DMY-treated rats received daily administration of DMY, 5 µg/kg dissolved in DMSO through the tail vein for 7 days. On the 7th day, rats in the DMSO and DMY groups were injected with 10 mg/kg LPS (Sigma-Aldrich, St. Louis, MO) through the tail vein. After 24 h, the animals were sacrificed and blood samples were transferred to tubes and immediately centrifuged (3000 rpm for 10 min at 4 °C). Serum samples were frozen and stored at –80 °C for biochemical analyses.

In vivo measurement of the mean arterial pressure

On the 8th day, catheters for invasive arterial blood pressure monitoring were inserted into the caudal ventral artery. First, the rat was placed in the supine position, and the tail skin was sterilized with povidone-iodine. Then, a 24-gauge catheter (Terumo, Tokyo, Japan) was aseptically inserted into the caudal ventral artery to allow blood pressure measurement. After backflow from the catheter was confirmed, the catheter was connected to the introducer of the arterial line. Visualization of the arterial waveform on the monitor was confirmed, and the catheter was flushed with heparinized physiological saline.

Artery isolation and in vitro vascular reactivity protocol

Thoracic aortas were isolated and prepared for vascular function studies (Spradley et al. 2012). On the 8th day, rats were anesthetized using 300 mg/kg chloral hydrate and decapitated. The thoracic aorta was carefully excised and placed in a Petri dish filled with cold Krebs solution (KHS) containing (in mM) NaCl 118.5, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5 and glucose 5.5 at 37 °C continuously bubbled with a 95% O_2 to 5%

CO_2 mixture (pH 7.4). The aorta was cleaned of excess connective tissue and cut into rings of approximately 3 mm in length. Thoracic aorta segments were mounted on two parallel stainless-steel pins for arterial isometric tension recording through a MAP2000 isometric force transducer (Alcott Biotech Co. Ltd., Shanghai, China) connected to a computer. Segments were suspended in an organ bath containing 20 mL of KHS and subjected to a tension of 2 g which was readjusted every 30 min during a 120 min equilibration period before drug administration. The vessels were then exposed to KCl (60 mM) to check their functional integrity. After washing out the thoracic aorta rings with KHS solution, we recorded the basal vascular tone prior to evaluating the contractile response by measuring the maximal peak height which is expressed as the maximal tension % achieved in response to 140 mM K^+ (K_{max}). Dose-response curves for NE (doses from 10^{-9} to 10^{-6} M) were obtained in aortic rings in a cumulative manner. To explore the role of K^+ channels in vascular tension, the contractility was quantitated after administration of 3×10^{-3} M TEA (tetraethylammonium, a nonselective potassium channel blocker), 3×10^{-8} M charybdotoxin (ChTX, a potent MaxiK channel blocker), 3×10^{-8} M glibenclamide (Glib, K_{ATP} channel blocker) and 3×10^{-3} M 4-AP (4-aminopyridine, a potent K_v channel blocker) and 3×10^{-3} M $BaCl_2$ (a potent Kir channel blocker).

Aortic smooth muscle cells (ASMCs) isolation and electrophysiological recording

ASMCs were isolated from the rat thoracic aorta by enzymatic digestion. Growth of passage 1–2 ASMCs was arrested for 24 h in serum-free Dulbecco's modified Eagle's medium before electrophysiological experiments. Single cells were released using a fire-polished pipette and allowed to adhere to the bottom of a recording chamber (0.5 mL). All operations were performed at room temperature (20–25 °C). Whole cell patch clamp recording was carried out with an Axopatch 700B amplifier (Axon Instruments, SV, San Francisco, USA). The potential of the membrane was clamped at –60 mV, subsequently digitized at 10–50 kHz (Digidata 1440 A interface, Axon Instruments). The resistance of patch electrodes was 4–5 MΩ. The bath solution contained (in mM): 134 NaCl, 6 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose and 10 HEPES (pH 7.4). The pipette solution contained the following (in mM): 107 KCl, 1.0 $MgCl_2$, 1.9 $CaCl_2$, 10 HEPES, 5 EGTA, 25 KOH, 0.1 Na_2ATP , 0.1 NaADP and 0.1 LiGTP (pH 7.2 adjusted with KOH, free $Ca^{2+} \sim 100$ nM).

Transient outward BK_{Ca} currents were measured. A pre-pulse (0 mV, 100 ms) was followed by test pulses (400 ms) from –80 to +80 mV in 10 mV increments. Test solutions bathing the cytoplasmic face of the patch membrane contained (in mM): 145 N-methyl-D-glucamine (NMDG), 3 KCl, 0.6 $MgCl_2 \cdot 6H_2O$, 2.5 $CaCl_2 \cdot 2H_2O$, 10 HEPES, 10 glucose (pH adjusted to 7.4 with Tris-base and 300 mOsm). ChTX (100 nM) was added to extracellular solutions to isolate the ChTX sensitive BK_{Ca} currents (the control currents subtracted the ChTX non-sensitive currents).

Freshly isolated VSMCs were obtained for the recording of K_{ATP} currents at room temperature. With references to Koide et al. (2014), K_{ATP} currents were recorded at –60 mV. After a stable baseline was obtained, the extracellular solution was changed to 140 mM K^+ solution, and the '140 mM K^+ ' bath solution was made by iso-osmotic replacement of NaCl with KCl. Pinacidil (10 µM) and glibenclamide (10 µM) were routinely added to extracellular solutions, pinacidil was used to increase

the inward current, otherwise glibenclamide was used to inhibit the inward current, then the glibenclamide sensitive K_{ATP} currents were recorded.

Measurement of NO, MDA, 3-NT, IL-1 β and TNF- α in serum

The Elisa reagents kits were purchased from Jiancheng Biologic Company (Nanjing, China). The method for plasma nitrite and nitrate levels (as a measure of NO) was based on the Griess reaction. Total nitrite was measured by spectrophotometry at 545 nm after conversion of nitrate to nitrite by copperized cadmium granules. MDA, the oxidative stress (OS) product of lipid peroxidation reacts with thiobarbituric acid under acidic conditions at 95°C to form a pink-colour complex with an absorbance at 532 nm. Rat serum 3-nitrotyrosine (3-NT), the OS product of proteins and the proinflammatory cytokines (TNF- α and IL-1 β) levels were measured, using a microplate reader (Thermo Multiskan MK3) at 450 nm.

Statistical analysis

Quantitative data are presented as mean \pm SEM. Statistical analysis was performed using SPSS (version 15, SPSS Inc., Chicago, IL) software. The basal vascular tension and serum ROS level in the control, LPS and LPS + DMY groups were analyzed using one-way ANOVA after significance is verified by the Dunnett's multiple comparison tests. Then, a two-way repeated measures ANOVA was used to compare the response to different doses of NE, with again Holm-Bonferroni *post hoc* tests if a significant difference emerged. Independent-sample *t*-test was used to assess differences between LPS and LPS + DMY groups. * $p < 0.05$ was considered statistically significant.

Results

Animals in Con + NS, Con + DMY, LPS + NS and LPS + DMY groups did not show any alteration in their general status under the LPS and DMY injection conditions. On the 7th day after the surgery, there was no significant difference in the mean weight of the rats among Con + NS, Con + DMY, LPS + NS and LPS + DMY groups.

DMY administration ameliorated LPS-induced vascular hyporesponsiveness in vivo

The percentage increase in mean arterial pressure (MAP) after NE or AVP administration in LPS + NS rats was much lower than that in Con + NS rats (3 μ g/kg NE: 15.2 ± 5.2 vs. $26.1 \pm 2.1\%$, $p < 0.05$, Figure 1(A); 3 U/kg AVP: 9.6 ± 3.0 vs. $18.0 \pm 5.5\%$, $p < 0.05$, Figure 1(B)). The percentage increase in MAP induced by vasopressor agents was raised by DMY injection (LPS + DMY vs. LPS + NS, NE: 20.4 ± 8.5 vs. $15.2 \pm 5.2\%$, $p < 0.05$, Figure 1(A); AVP: 14.1 ± 2.5 vs. $9.6 \pm 3.0\%$, $p < 0.05$, Figure 1(B)). There was no significant difference between the Con + NS and Con + DMY group (NE: 26.1 ± 2.1 vs. $25.0 \pm 6.0\%$, $p < 0.05$, Figure 1(A); AVP: 18.0 ± 5.5 vs. $17.2 \pm 6.0\%$, $p < 0.05$, Figure 1(B)).

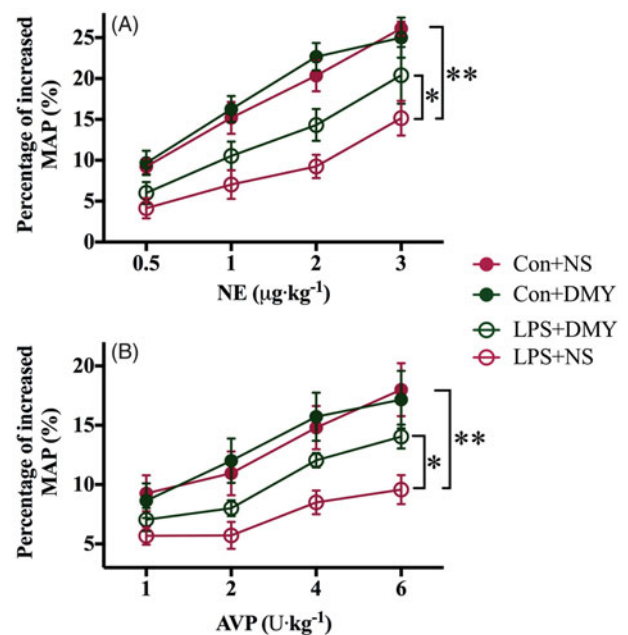


Figure 1. Dihydromyricetin significantly ameliorated the vascular hyporesponsiveness induced by LPS. (A) The % of increasing mean arterial pressure (MAP) of control and LPS mice with or without dihydromyricetin after different doses of norepinephrine (NE) administered (0.5, 1, 2, 3 μ g/kg, $n = 6$). (B) The percentage of increase in MAP of control and LPS rats with or without dihydromyricetin after different doses of arginine vasopressin (AVP) administered (1, 2, 4, 6 U/kg, $n = 6$). The percentage of increase in mean arterial pressure: (increased MAP – basal MAP)/basal MAP. Data are presented as mean \pm SEM. A two-way repeated measures ANOVA was used, with again Holm-Bonferroni *post hoc* tests if a significant difference emerged. *represents $p < 0.05$, ** $p < 0.01$.

MaxiK and K_{ATP} channels mediated the DMY-induced improvement of vasoconstrictor hyporeactivity in experimental sepsis

The basal vascular tone of the thoracic aorta rings isolated from the rats with LPS administration decreased markedly, however, DMY could reverse it (Control: 2.2 ± 0.4 g; LPS: 1.5 ± 0.2 g; LPS + DMY: 2.0 ± 0.4 g, Figure 2(A)). The contractile response for arterial strips to NE (3×10^{-6} M) in LPS + NS rats was significantly blunted compared with that in Con + NS rats (NE: 1.8 ± 0.9 vs. 3.7 ± 1.1 g, $p < 0.05$, Figure 2(B); AVP: 4.1 ± 0.3 vs. 3.9 ± 0.1 g, $p < 0.05$, Figure 2(B)). The vascular hyporesponsiveness to vasopressor agents was improved by DMY injection (LPS + DMY vs. LPS + NS, NE: 2.7 ± 0.8 vs. 1.8 ± 0.9 g, $p < 0.05$, Figure 2(B)).

NE induced contraction divided by potassium-induced maximum contraction (K_{max}) (NE-induced contraction/ K_{max}) was adopted to indicate the shrink ability of the isolated arteries. To explore which potassium channels played a pivotal role in DMY-induced improvements of vascular hyporeactivity, isolated arteries were pretreated with potassium channel blockers previous to NE incubation. After pretreatment of TEA (tetraethylammonium, a nonselective potassium channel blocker, 3×10^{-3} M), the improvement of vasoconstrictor hyporeactivity induced by DMY was significantly blocked (10^{-6} M NE: LPS + NS 70.3 ± 9.6 vs. LPS + DMY $66.8 \pm 17.7\%$, Figure 2(C)). Meanwhile, the contribution of MaxiK, K_{ATP} , Kv and Kir channels to DMY induced amelioration of vasoconstrictor hyporeactivity was examined. ChTX (a specific MaxiK channel blocker) and Glib (a specific K_{ATP} channel blocker) pretreatment could also diminish the DMY-induced improvement of vasoconstrictor

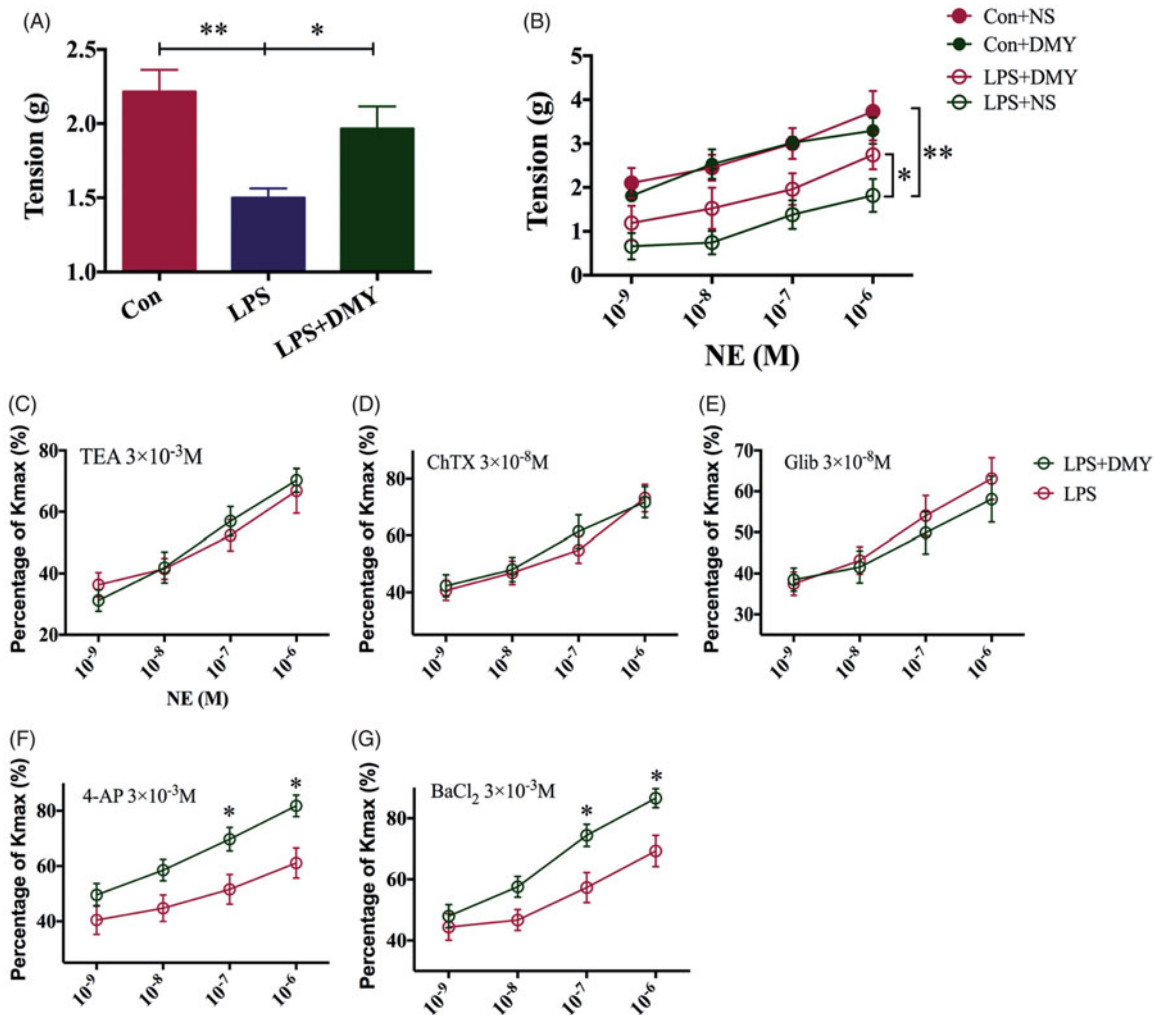


Figure 2. MaxiK and K_{ATP} channels in vascular smooth muscle cells played a critical role in vasoconstrictor hyporeactivity of LPS rats. (A) The basal vascular tone of thoracic aorta rings isolated from control, LPS and LPS + DMY groups ($n = 6$). The data were analyzed by using one-way ANOVA if significant by Dunnett's multiple comparison tests. (B) The contractile response for arterial strips from control and LPS rats with or without DMY to norepinephrine (3×10^{-5} M, $n = 6$). (C) NE contractions with TEA pretreatment were expressed as % of maximal contraction elicited with K in LPS and LPS + DMY rats ($n = 6$). (D) NE contractions with ChTX pretreatment were expressed as % of maximal contraction elicited with K in LPS and LPS + DMY rats ($n = 6$). (E) NE contractions with Glib pretreatment were expressed as % of maximal contraction elicited with K in LPS and LPS + DMY rats ($n = 6$). (F) NE contractions with 4-AP pretreatment were expressed as % of maximal contraction elicited with K in LPS and LPS + DMY rats ($n = 6$). (G) NE contractions with BaCl₂ pretreatment were expressed as % of maximal contraction elicited with K in LPS and LPS + DMY rats ($n = 6$). Data were presented as mean \pm SEM. A two-way repeated measures ANOVA was used, with again Holm-Bonferroni *post hoc* tests if a significant difference emerged. *represents $p < 0.05$, ** $p < 0.01$.

hyporeactivity (ChTX: LPS + NS 73.2 ± 11.8 vs. LPS + DMY $71.8 \pm 13.5\%$; Glib: LPS + NS 63.1 ± 12.5 vs. LPS + DMY $58.1 \pm 13.7\%$, Figure 2(D,E)), while the same effect was not observed in 4-AP (4-aminopyridine, a specific Kv channel blocker) and BaCl₂ pretreatment (barium chloride, a specific Kir channel blocker) used (4-AP: LPS + NS 81.8 ± 9.6 vs. LPS + DMY $61.1 \pm 13.2\%$; BaCl₂: LPS + NS 86.6 ± 7.6 vs. LPS + DMY $69.3 \pm 12.6\%$, Figure 2(F,G)).

DMY blunted the high sensitized MaxiK and K_{ATP} channels of ASMCs isolated from the thoracic aorta of LPS rats

ASMCs were isolated from the thoracic aorta by using enzymatic digestion to obtain highly purified acute isolated ASMCs. Figure 3(A) illustrated that under the same experimental conditions, the whole-cell MaxiK currents density in LPS + DMY was smaller than that in LPS rats. The statistical analysis was shown in Figure 3(C) (0.2 ± 0.02 vs. 0.1 ± 0.02 nA/pF, $p < 0.01$). The whole

cell recordings were carried out in a symmetrical 140 mM K⁺ solution to optimize the recordings, and the cells were held at a holding potential of -60 mV. Raising the extracellular K⁺ to 140 mM induced small K_{ATP} currents. Pinacidil (10 μ M) was applied to increase an inward current in cells from LPS and LPS + DMY rats for enhancing the K_{ATP} currents (Figure 3(B)) and glibenclamide (a K_{ATP} channel inhibitor) could revert it in both cell types. Then pinacidil-induced K_{ATP} currents in isolated ASMCs from both LPS and LPS + DMY models were obtained. The magnitude of the K_{ATP} current in ASMCs from LPS + DMY rats was significantly lower than those from LPS rats (-26.7 ± 3.0 vs. -16.9 ± 3.7 pA/pF, Figure 3(D), $p < 0.05$).

DMY decreased the serum concentrations of cytokines and oxidative stress increased by LPS injection

The serum level of NO, MDA, IL-1 β and TNF- α significantly increased in LPS + NS rats compared with those in control rats

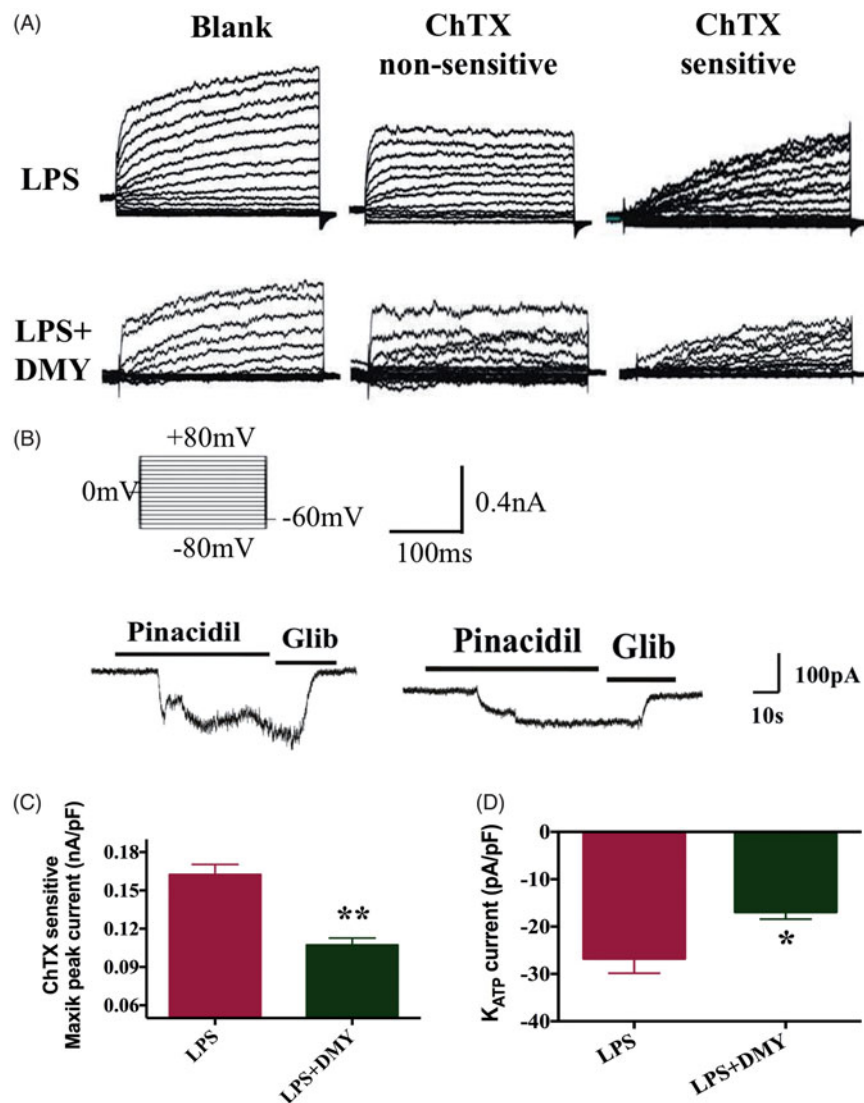


Figure 3. MaxiK and K_{ATP} currents in ASMCs from LPS and LPS + DMY rats. The whole-cell K⁺ currents in aortic myocytes measured by conventional whole-cell patch-clamp technique. (A and C) Typical recordings of MaxiK currents in ASMCs from LPS and LPS + DMY rats, respectively. In the group of LPS + DMY, BK_{Ca} peak currents were significantly smaller at the voltage -60mV, compared with those in LPS group ($n=8$, $*p < 0.05$). (B) K_{ATP} currents of ASMCs from LPS and LPS + DMY rats. 10 μ M pinacidil was administered to increase an inward current, and then it was reverted by glibenclamide to obtain pinacidil-induced K_{ATP} currents in ASMCs from both LPS and LPS + DMY models. (D) The magnitude of K_{ATP} current in ASMCs from LPS + DMY rats was significantly smaller than that from LPS rats ($n=6$, $*p < 0.05$). Data are presented as mean \pm SEM. Independent-sample *t*-test was used to assess differences between LPS and LPS + DMY groups.

(NO: 25.8 ± 3.6 vs. 8.6 ± 1.9 ; MDA: 20.1 ± 2.1 vs. 5.1 ± 1.0 ; IL-1 β : 68.5 ± 6.0 vs. 45.5 ± 7.7 ; TNF- α : 26.3 ± 3.1 vs. 14.5 ± 1.7). However, the serum level of ROS was significantly reversed by DMY (NO: 14.9 ± 1.6 ; MDA: 12.5 ± 1.4 ; IL-1 β : 50.7 ± 4.5 ; TNF- α : 15.5 ± 1.5). There was no significant difference in the serum level of 3-NT among the Con + NS, Con + DMY, LPS + NS and LPS + DMY (Figure 4).

Discussion

The principal findings of this work were that DMY administration improved vascular hyporesponsiveness in LPS induced sepsis, via attenuating the over-excited MaxiK and K_{ATP} channels and free radical scavenging, this is supported by the following observations: (1) DMY administration ameliorated LPS-induced vascular hyporesponsiveness, represented by increased MAP; (2) MaxiK and K_{ATP} channels mediated the DMY produced improvement of vasoconstrictor hyporeactivity in experimental sepsis; (3) DMY blunted the highly sensitized MaxiK and K_{ATP}

channels of ASMCs isolated from the thoracic aorta of LPS rats; (4) DMY decreased the degree of oxidative stress induced by LPS injection. Taken together, we verified for the very first time that MaxiK and K_{ATP} channels mediated the improvements of vascular hyporesponsiveness in LPS injected rats.

It has been reported that DMY exhibited antioxidant capacity via increasing the activity of heme oxygenase-1 (HO-1) (Kou et al. 2012) and the acid-fast activities of DMY were mainly attributed to its *ortho*-trihydroxy group (Xin et al. 2012). A previous study suggested that DMY inhibited T cell activation and secretion of related cytokines by binding to the 46th cysteine of IKK β and inhibiting IKK β kinase activity (Xin et al. 2012). The important immune cells, macrophages and T cells, play a pivotal role in oxidative stress of septic shock. Macrophages are not only involved in the inflammatory response, but also participate in the secretion of inflammatory factors COX-2, iNOS, TNF- α and IL-1 β (Won et al. 2006). It has also been reported that DMY can inhibit the release of iNOS, IL-6 and other inflammatory factors. This effect can be attributed to inhibition of IKK β , IKB α and

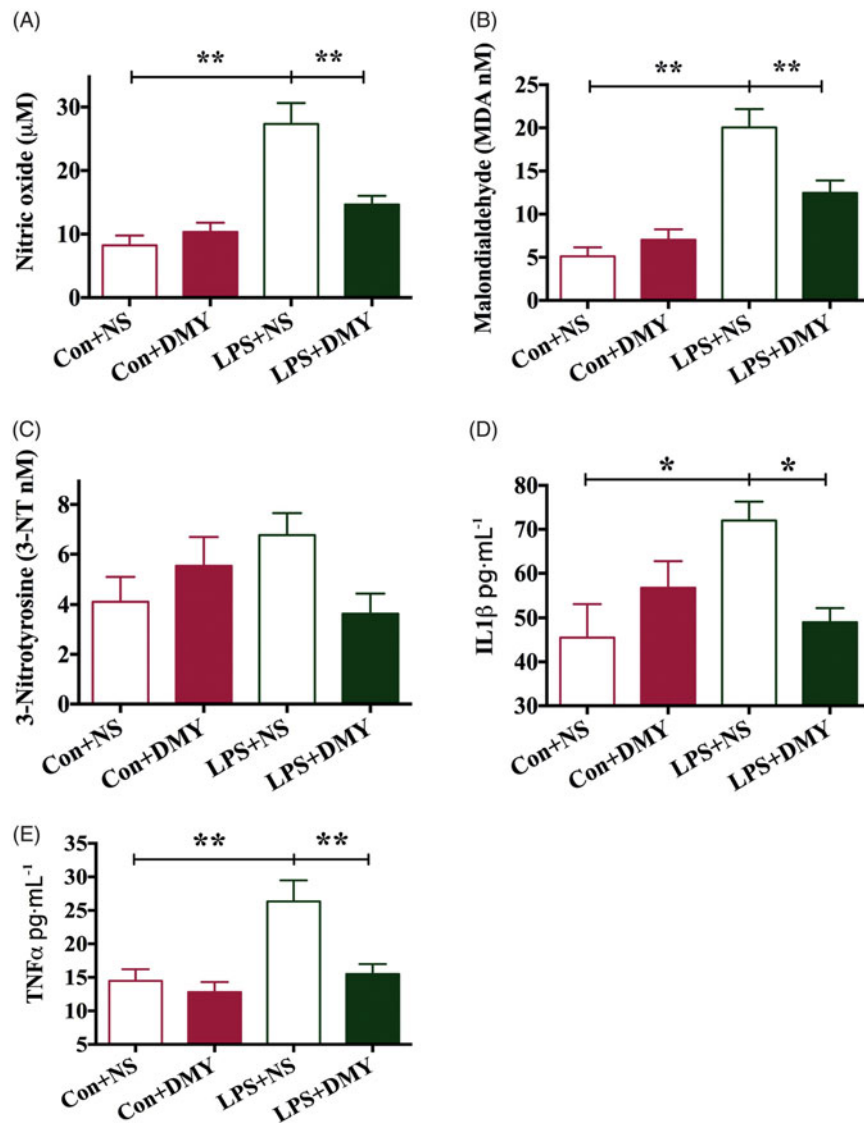


Figure 4. Levels of NO, MDA, 3-NT, IL-1 β and TNF- α in serum from the control group, CON + DMY group, LPS + NS group and LPS + DMY group ($n=6$). Data are presented as mean \pm SEM. The data were analyzed by using one-way ANOVA if significant by Dunnett's multiple comparison tests. * $p < 0.05$, ** $p < 0.01$.

NF- κ B signal transduction pathways (Hou et al. 2015). In this study, we observed the preventive effects of DMY on LPS-induced vascular hyporesponsiveness in rats. The results showed that DMY could significantly relieve vascular hyporesponsiveness caused by LPS and this effect could be blocked by MaxiK and K_{ATP} inhibitors incubation previous to NE treatment, which suggests that DMY may inhibit the inflammatory response, thus reverse the over-excited MaxiK and K_{ATP} channels in VSMCs.

Accumulating evidence shows that the activity of ion channels, especially the potassium channel on ASMCs, plays a pivotal role in affecting the contractile state of the peripheral arteries. Excess opening of the potassium channel in ASMCs caused membrane hyperpolarization of ASMCs resulting in vascular hyporeactivity (Brayden 2002; Chrissobolis and Sobey 2003). After pretreatment with MaxiK and K_{ATP} inhibitors, the improvement of vasoconstrictor hyporeactivity induced by DMY was significantly blocked, this indicated that MaxiK and K_{ATP} channels mediated the DMY-induced improvement of vasoconstrictor hyporeactivity. The compromised ability of an artery to constrict is likely to be caused by the defective function of the potassium channel in blood vessels, and it may be due to a

change in unitary conductance, or open probability of the channels, and a change in expression number (Karabacak et al. 2015). We detected the currents of MaxiK and K_{ATP} by using electrophysiological methods. DMY blunted the high sensitized MaxiK and K_{ATP} channels of ASMCs isolated from the thoracic aorta of LPS rats.

Vasoplegia is thought to be a key aspect in the pathogenesis of cardiovascular alterations during severe sepsis and a key factor responsible for the death of patients with septic shock, due to the persistent and irreversible hypotension (Lundy and Trzeciak 2009). Oxidative stress during severe sepsis is one of the important factors resulting in vascular hyporeactivity to vasoconstrictors (Gamcrldize et al. 2015). Inactivation of α -adrenoceptors by peroxynitrite may be a possible mechanism of cardiovascular hyporeactivity to catecholamines and systemic hypotension in sepsis (Shintani et al. 1996). It has been demonstrated that peroxynitrite scavenging improves contractile responses in aorta and microvasculature and has protective effects from vascular dysfunction in the sepsis, which is consistent with our results. Furthermore, some researchers proved that over-activation of K_{ATP} channels also participated in the vascular hyporeactivity to vasoconstrictors

in sepsis (Rodrigo and Standen 2005; Sordi et al. 2011). Normally channel opening at the plasma membrane promotes K^+ loss from the cell and maintenance of membrane resting potential. In vascular smooth muscle, potassium channels are extensively regulated by signalling pathways and cause vasodilation, contributing to both resting blood flow and vasodilator-induced increases in flow. Excessive activation of K^+ channels on VSMCs membranes leads to membrane hyperpolarization, and the inhibition of Ca^{2+} entry through voltage-gated Ca^{2+} channels, thereby inducing cell relaxation, vasodilatation, and eventually resulting in hypotension and vascular hyporeactivity.

The relationship between potassium channels and oxidative stress has been established. Potassium channels can be over-activated by hypoxia, acidosis, hyperlactatemia, NO and peroxynitrite. Soh et al. (2001) reported that in neonatal rat hippocampal neurons, the reducing reagent glutathione (GSH) increased BK channel activity, whereas its oxidized form (GSSG) had the opposite effect. This suggests a redox modulatory mechanism when GSSG was applied to the intracellular side of the cell membrane. However, Zhang and Horrigan (2005) reported that after intracellular application of the oxidizing agent DTNB, there is an increase in open times and decrease in closing times of BK channels from adult native hippocampal CA1 pyramidal neurons. On the other hand, GSH had no apparent effect on BK channel activity.

The present study has some limitations. First, our experiments were performed in rats, and we did not have clinical data to support our conclusion. Second, considering its close relationship with blood pressure regulation, mesenteric artery strips, instead of thoracic aortic rings, it should be used to explore the vascular hyporeactivity of LPS and LPS + DMY rats. Finally, it would be desirable to silence or overexpress the MaxiK and K_{ATP} genes in order to explore the reasons for channel sensitization. A definitive causal relationship between MaxiK, K_{ATP} and DMY-induced improvement of vascular hyporeactivity could be drawn if the DMY-induced improvement of vascular hyporesponsiveness could be obviously inhibited after MaxiK and K_{ATP} channels expression changed.

In summary, our results indicate that the impaired contractility of ASMCs in experimental sepsis could be ameliorated by DMY administration. Such an effect is mediated by the normalization of the over-excited MaxiK and K_{ATP} channels, possibly via the inhibition of oxidative stress.

Disclosure statement

The authors have declared no conflict of interest.

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